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SHORT COMMUNICATION

Meiotic studies in *Brachistosternus alienus* (Scorpiones; Bothriuridae)

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Abstract. *Brachistosternus* Pocock 1893 is the most diverse genus of the scorpion family Bothriuridae. Only four species of the genus have been cytogenetically analyzed so far. We report herein the cytogenetic analysis of *Brachistosternus alienus* Lönnberg 1898 from Comallo (Río Negro province, Argentina). This species is widely distributed in the Monte phytogeographic province, located in central and northern Argentine Patagonia. Meiotic cells of *B. alienus* from Comallo show 23 homomorphic achiasmatic bivalents. The karyotype of this species contains scarce AT-rich regions that may be associated with the heterochromatin of centromeric regions. Giacomozzi (1977) reports $n = 14$ for *B. alienus* from Chubut province. Unfortunately, it is not presently possible to determine if those specimens correspond to *B. alienus* or to a sympatric species, *Brachistosternus angustimanus* Ojanguren-Affilastro & Roig-Alsina 2001. These different chromosome numbers of the two populations analyzed may reflect the occurrence of a chromosomal polytypism in *B. alienus*, or they may characterize different species.

Keywords: Cytogenetics, DAPI staining, karyotype, meiosis, scorpion

In recent years, the aim of our research group has been to study cytogenetics in scorpions belonging to the family Bothriuridae Simon 1880. The genus *Brachistosternus* Pocock 1893 is of particular concern because its species-level taxonomy is fairly well resolved (Rodríguez Gil et al. 2009). Moreover, the phylogeny of this group has been recently evaluated (Ojanguren-Affilastro & Ramírez 2009), providing a more comprehensive evolutionary-based framework for cytogenetic studies.

Brachistosternus is the most diverse genus of the family Bothriuridae, comprising 40 of the ~150 known species of the family. Its species inhabit arid and semi-arid areas of western and southern South America, from Ecuador to southern Patagonia, Argentina (Cekalovic 1969; Ojanguren-Affilastro 2001, 2003a, b, 2005a, b; Ochoa & Ojanguren-Affilastro 2007; Ojanguren-Affilastro & Scioscia 2007; Ojanguren-Affilastro et al. 2007a, b). The group's tremendous diversification is relatively recent, and may have resulted from the onset of aridity in the late Miocene (7–10 mya); most of the basal bothriurid genera occur in mesic environments (Prendini 2003).

To date, there are few published cytogenetic studies on bothriurids, comprising only ten species (Piza de Toledo 1947; Ferreira 1968; Giacomozzi 1977; Rodríguez Gil et al. 2009; Schneider et al. 2009a). Four of these species belong to the Argentine representatives of the genus *Brachistosternus*. *Brachistosternus (Ministernus) ferrugineus* (Thorell 1876) and *Brachistosternus* (*Brachistosternus*) *montanus* Roig Alsina 1977 have $n = 23$ (Rodríguez Gil et al. 2009). *Brachistosternus* (*B.*) *pentheri* Mello-Leitão 1931 shows two different cytotypes, $n = 23$ and $n = 21$, which correspond to two slightly different morphs (Rodríguez Gil et al. 2009). Finally, the haploid karyotype of *Brachistosternus* (*B.*) *alienus* Lönnberg 1898 is reported to consist of 14 chromosomes (Giacomozzi 1977).

Brachistosternus alienus is widely distributed in the Monte phytogeographic province, located in central and northern Argentine Patagonia. A taxonomic revision of this species (Ojanguren-Affilastro 2001) revealed that geographically distant populations exhibit some morphological differences, raising doubts about their identity. There are no data on the cytogenetics of this species except for the chromosome number of specimens from the Patagonian province of Chubut (Giacomozzi 1977); however, this information is incomplete and species identity of these specimens is uncertain.

In this study, two males of *B. alienus* belonging to a population from a locality near Comallo (Río Negro province) in northern Argentine Patagonia (41°03′01″S, 70°26′51.7″W) were examined. Comallo is located about 500 km from the type locality in Chubut province. The karyotype and meiotic behavior of chromosomes was described and furthermore, the distribution of the AT-rich regions in a representative of the genus *Brachistosternus* was determined for the first time.

The males of *B. alienus* were collected at night using UV lamps, then were carried to the laboratory and killed by cooling to −20°C. Their gonads were dissected in saline solution (0.154 M NaCl), incubated in hypotonic solution (1:1 saline solution:distilled water, 30 min), fixed in a freshly prepared mixture of ethanol:chloroform:acetic acid (6:3:1, 30 min), and stored in fresh fixative mixture. Pieces of testis were placed on slides and dissociated in a drop of 80% acetic acid with tungsten needles. Preparations with a drop of suspension were placed on a heated histological plate (approximately 45°C); the suspension was spread on the slides using a tungsten needle. Finally, the preparations were air-dried and stained with 5% Giemsa solution in distilled water for 10 min. Following light microscopy observations, the slides were destained using a mixture of ethanol:acetic acid (3:1) for 2 h and stained with fluorescent dye DAPI (4′-6-diamidino-2-phenylindole) to detect blocks of AT-rich regions. Briefly, the slides were rinsed twice with 4xSSC buffer for 10 min and air-dried. One drop of 0.5 µg/ml DAPI in PBS (phosphate buffered saline) containing 1% Triton X-100 was placed on each slide, covered with a coverslip and incubated in a moist chamber (30 min, room temperature). Following coverslip removal, the slides were rinsed with 4xSSC and air-dried. Finally, they were mounted with 50 µl of VECTASHIELD® (Vector Laboratories, Inc.), covered by a coverslip, and stored at 4°C overnight before microscopic analysis. The staining with DAPI and air-drying steps were performed in the dark.

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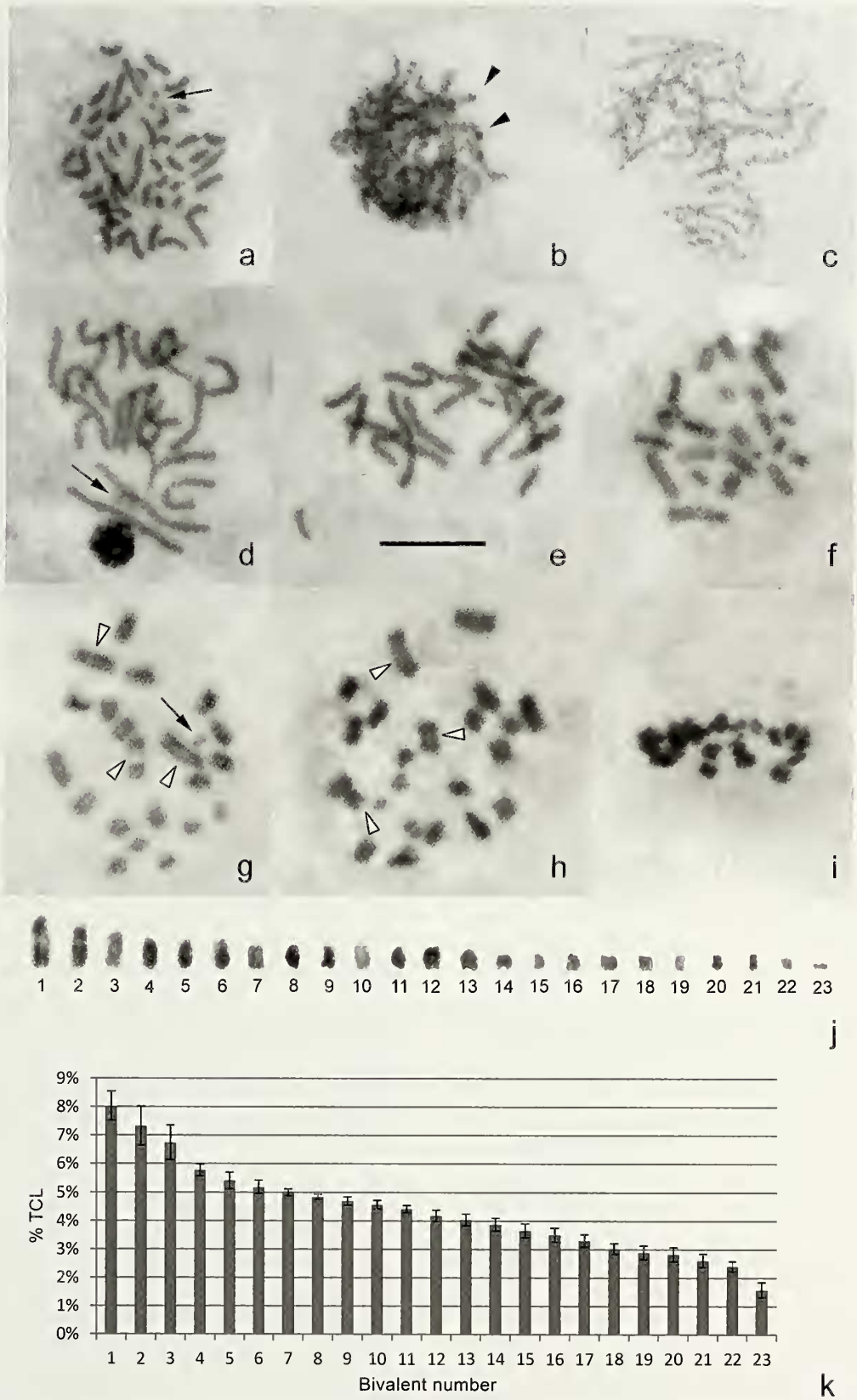

Figure 1.—Spermatogenesis, meiotic karyogram and ideogram of *Brachistosternus alienus* ($2n = 46$, $n = 23$). a. Early spermatogonial metaphase; b. Zygotene; c. Pachytene; d. Early postpachytene; e. Middle postpachytene; f. Late postpachytene; g,h. Prometaphase I; i. Metaphase I; j. Meiotic karyogram (based on prometaphase bivalents depicted in 1g); k. Meiotic idiogram. The arrows point to the smallest pair. The black arrowheads point to the positively heteropycnotic telomeric regions. The white arrowheads mark the increase in separation of the homologous chromosomes. Scale = 10 μm.

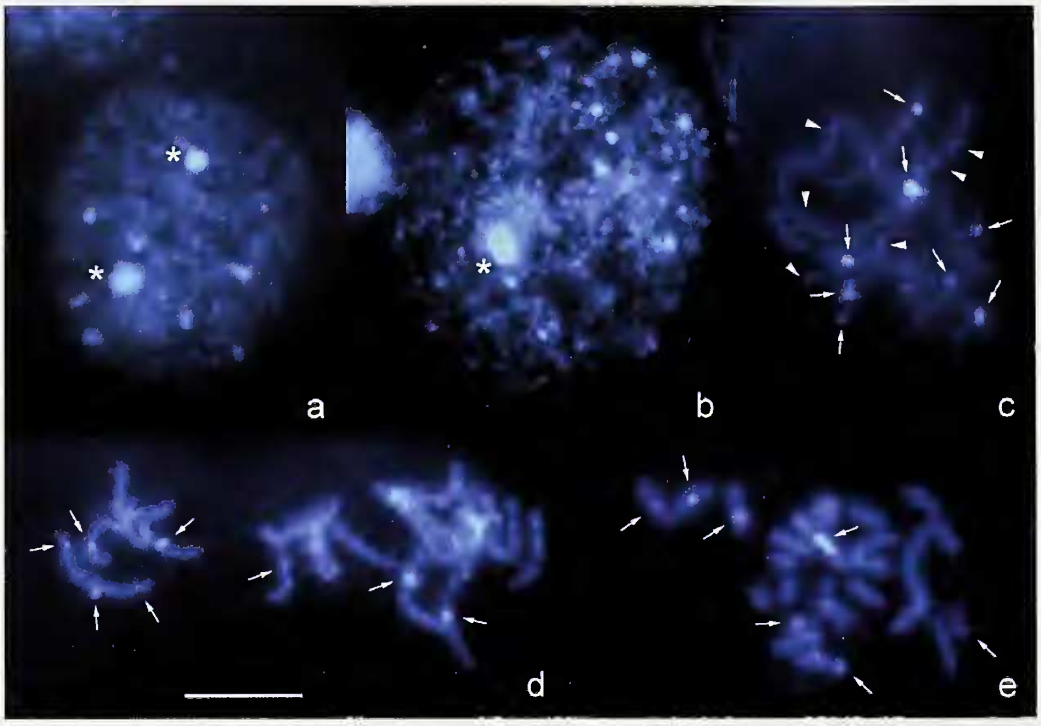

Figure 2.—DAPI staining of interphase nuclei and meiotic chromosomes of *Brachistosternus alienus*. a. Interphase; b. Leptotene-zygotene; c. Late pachytene; d. Early postpachytene; e. Late postpachytene. Asterisks point to chromocenters. Arrows point to DAPI-positive bands showing higher intensity of fluorescence. Arrowheads point to DAPI-positive bands exhibiting lower intensity of fluorescence. Scale = 10 μm.

To determine the meiotic karyotype, chromosome measurements were performed in 11 well-spread postpachytene and prometaphase I using Micro-Measure software, version 3.3 (Reeves & Tear 2000). The relative length of each bivalent was calculated as a percentage of total complement length (TCL; length of all bivalents of the karyotype). These data allowed us to construct an ideogram. Bivalents of seven prometaphase I exhibiting the same degree of condensation were measured to determine absolute length (μm) of each bivalent.

The spermatogonial mitosis shows 46 monocentric chromosomes; one pair is remarkable for its small size (Fig. 1a). During zygotene, terminal regions of some bivalents exhibit small positively heteropycnotic blocks (Fig. 1b), which are no longer distinguishable at pachytene (Fig. 1c). Twenty-three bivalents are seen from late pachytene until prometaphase I (Fig. 1d–h). During the chromatin condensation, at early and middle postpachytene the homologous chromosomes of the bivalents remain tightly joined (Fig. 1d, e). At late postpachytene it can be seen that the homologous chromosomes lie parallel to each other, thus showing the absence of chiasmata, which is a common feature of meiosis in male scorpions (Fig. 1f). At prometaphase I and metaphase I, many bivalents show a small region in the intercalary or terminal position where the homologues are more separated (Fig. 1g–i).

The meiotic karyotype shows 23 homomorphic bivalents (Fig. 1j). Three groups of bivalents can be identified as follows: three large bivalents of different size (4.58, 4.08 and 3.73 μm), 19 middle-sized bivalents decreasing gradually in size (3.30, 3.13, 3.02, 2.92, 2.83, 2.73, 2.66, 2.59, 2.45, 2.38, 2.28, 2.16, 2.08, 1.95, 1.79, 1.76, 1.73, 1.57 and 1.44 μm), and the smallest bivalent of the complement (0.94 μm). The relative length of the bivalents ranges from 8.03% to 6.74% in the first group, from 5.77% to 2.41% in the second group, and is 1.59% for the smallest bivalent (Fig. 1k).

Fluorescent banding using DAPI revealed AT-rich regions, namely chromocenters at interphase nuclei as well as bands on bivalents at prophase of the first meiotie division. These regions vary in size and number. They are presumably formed by AT-rich constitutive heterochromatin. Interphase and leptotene/zygotene nuclei usually contain one or two conspicuous DAPI-positive chromocenters as well as some signals of intermediate or low intensity (Fig. 2a, b). Pachytene karyotypes exhibit between eight and 10 intercalary DAPI-positive bands showing higher intensity of fluorescence and some bands of lower intensity in terminal or intercalary regions (Fig. 2c). During the following bivalent condensation, most of the low-intensity bands become indistinguishable (Fig. 2d). At late postpachytene, intercalary AT-rich bands can be identified in one large and four middle-sized bivalents, while terminal bands are detected in two middle-sized bivalents (Fig. 2e).

Heterochromatin distribution has been analyzed in only a few scorpions so far, namely eight species of Buthidae (belonging to genera *Isometroides*, *Isometrus*, *Lychas* and *Tityus*) (Shanahan 1989a; Schneider et al. 2009b; Schneider & Cella 2010), six species of Urodacidae (genus *Urodacus*) (Shanahan 1989b), and two species of Bothriuridae belonging to the genus *Bothriurus* (Schneider et al. 2009a). Heterochromatin content and distribution in Buthidae is variable. In contrast to the other scorpions, this family shows holokinetic chromosomes. Most heterochromatin of buthids occurs at telomeric regions. Shanahan (1989b) reports pericentromeric bands in most *Urodacus* species studied. In *Bothriurus rochensis* Schneider et al. (2009a) report small C-bands in the centromeric regions of some subtelocentric and submetacentric pairs, as well as in the terminal region of the long arm of several submetacentric pairs. These authors also described AT-rich bands in the terminal regions of two pachytene bivalents of *B. rochensis*. In contrast, heterochromatin has not been detected by C-banding and AT specific fluorochrome in *Bothriurus araguayae* (Schneider et al. 2009a). Blocks of presumed AT-rich heterochromatin of *Brachistosternus alienus* may correspond to pericentromeric regions of monobrachial and bibrachial chromosomes.

For *Brachistosternus* species evaluated so far, the haploid set of *B. ferrugineus* and *B. montanus* is formed by 23 chromosomes. The same number is found in populations of *B. pentheri* from the northernmost

limit of the species distribution (north of La Rioja province, Argentina) (Rodríguez Gil et al. 2009). Individuals of *B. pentheri* from northern populations are larger and much less pigmented than the typical morph. On the other hand, populations corresponding to the holotypic morph, which are distributed from the south of La Rioja province to the southeast of Buenos Aires province, have n = 21 (Giacomozzi 1977; Rodríguez Gil et al. 2009). Rodríguez Gil et al. (2009) propose that marginal populations of *B. pentheri* from northern Argentina could be considered a subspecies or even a different species due to their specific morphological features and different chromosome number.

The haploid karyotype of *B. alienus* from Comallo (province of Río Negro, Argentina) (this study) comprises 23 chromosomes, whereas the specimens of *B. alienus* studied by Giacomozzi (1977) have n = 14. Giacomozzi (1977) stated that the specimens were determined by Dr. E. Maury and sampled in the province of Chubut, Argentina. Although further information on the collection site is missing, data from that period suggests that the specimens were collected near Puerto Madryn, the species' type locality. However, it is impossible to determine if the specimens studied by Giacomozzi (1977) belong to *B. alienus* or to *B. angustimanus* Ojanguren-Affilastro & Roig-Alsina 2001 (a sympatric species), because the material is no longer available. At the time of Giacomozzi's study, most authors based the identification of *B. alienus* on a redescription of this species by Mello-Leitão (1934, 1945), which corresponds more closely to *B. angustimanus* than to the original description of *B. alienus* by Lönnberg (1898). Both species are sympatric over most of their geographic range. *Brachistosternus angustimanus* is larger and usually more abundant than *B. alienus* (Ojanguren-Affilastro 2001; Ojanguren-Affilastro & Roig-Alsina 2001; Rodríguez Gil et al. 2009). Conclusive evidence of species misidentification was provided by Maury (1972), who assigned a hemispermatophore of *B. angustimanus* to *B. alienus*.

The considerations mentioned above and the reduced chromosome number (n = 14) of specimens studied by Giacomozzi suggest that these specimens belonged to *B. angustimanus*. However, we cannot rule out the possibility that Giacomozzi's specimens correspond to *B. alienus*; in this case, species should be considered polytypic, like *B. pentheri* (Rodríguez Gil et al. 2009). The specimens analyzed in this work vary slightly in morphology from the typical morph, and Comallo is about 500 km from the type locality of *B. alienus* (the putative sampling site of Giacomozzi's specimens) (Ojanguren-Affilastro 2001, 2005b). At this time access to living material of either *B. alienus* or *B. angustimanus* from Puerto Madryn for cytogenetic analysis was not possible. Further studies involving individuals unequivocally identified as *B. alienus* and *B. angustimanus* from different localities are needed to establish whether variation in chromosome number reflects the occurrence of polytypism or different species.

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